SEASONAL OCCURRENCE AND MATURATION OF *NEOECHINORHYNCHUS RUTILI* (ACANTHOCEPHALA) IN BARBEL, *BARBUS BARBUS* (PISCES), OF THE JIHLAVA RIVER, CZECH REPUBLIC

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Summary :

From April 1992 until June 1993, the seasonal occurrence and maturation of the acanthocephalan *Neoechinorhynchus rutili* in its definitive host, the barbel *(Barbus barbus)*, was studied in the Jihlava River (the Danube basin), Czech Republic. The parasite occurred in barbel throughout the year (overall prevalence 84% and intensity of infection 1-533 [mean 25] acanthocephalans per fish), but there were distinct seasonal fluctuations in prevalence and mean intensity values: prevalence attained 100% in February and March, whereas its lowest value was recorded in July; the lowest values of mean intensity occurred in July-August. Both prevalence and mean intensity values distinctly increased with the body length of hosts. The parasite exhibited a distinct annual maturation cycle when egg-producing females were present mainly in the spring and summer (May - July) and, at a lesser extent, also from December to April of the next year. The seasonal maturation of *N. rutili* appears to be induced principally by the temperature regimen in the locality. *N. rutili* cystacanths were recovered from larval *Sialis lutaria* (Megaloptera) (prevalence 6%).

Résumé *:* OCCURENCE SAISONNIÈRE DE *N. RUTILI* CHEZ LE BARBEAU

D'avril 1992 à juin 1993, l'occurence saisonnière et la maturation de l'acantocéphale Neoechinorhynchus rutili *chez son hôte définitif, le barbeau* (Barbus barbus), *ont été étudiées dans la rivière Jihlava (bassin du Danube), République Tchèque. Le parasite était présent chez le barbeau tout au long de l'année (prévalence globale, 84%; intensité de l'infection, 1-533 [moyenne, 25] acantocéphales par poisson), mais les valeurs de la prévalence et de l'intensité moyenne ont nettement fluctué : la prévalence a atteint 100% en février et mars, alors que sa valeur la plus basse était observée en juillet; les valeurs les plus basses de l'intensité moyenne ont été observées en juillet-août. Les valeurs de la prévalence comme celles de l'intensité moyenne ont nettement augmenté avec la longueur corporelle de l'hôte. Le parasite a montré un cycle annuel de maturation différent lorsque les femelles productrices d'œufs étaient essentiellement présentes au printemps et en été (mai-juin), et, à un moindre degré, de décembre à avril de l'année suivante. La maturation saisonnière de* N. rutili *semble être principalement induite par le régime de température de la localité. Des cystacanthes de* N. rutili *ont été retrouvés à partir de larves de* Sialis lutaria *(Megaloptera) (prévalence de 6%).*

KEY WORDS : *Neoechinorhynchus rutili. Barbus barbus.* seasonality. maturation. *Sialis.* paratenic host.

MOTS CLES : Neoechinorhynchus rutili. Barbus barbus. *caractère saisonnier. maturation.* Sialis. *hote paraténique*

INTRODUCTION

The acanthocephalan *Neoechinorhynchus rutili* (Müller, 1780) is a widely distributed intestinal parasite of freshwater fishes in the holarctic region; it is known to occur in a large number of fish species of different families, but its principle hosts are cyprinids. The infection is often very high and it is generally considered to be pathogenic to its fish host (Bauer *et al.*, 1977). In the Czech Republic it occurs in all main basins, both in wild fish populations and pond-cultured fish.

The development of *Neoechinorhynchus rutili* is not well known. Larvae (cystacanths) of this parasite were recorded from the naturally infected leech *Erpobdella octoculata* (Robin, 1871), larval alder flies *Sialis lutaria* (Insecta : Megaloptera) (Villot, 1885; Robertson, 1953; Lassiere and Crompton, 1988), various species of ostracods (Ostracoda) (Brady, 1910; Jarecka, 1956; Styczynska, 1958; Merrit and Pratt, 1964; Walkey, 1967; Valtonen, 1979), and from crayfish *Pacifastacus trowbridgi* (Merrit and Pratt, 1964). The postembryonal development of *N. rutili* in the intermediate host (ostracods) was experimentally studied by Merrit and Pratt (1964) in North America. Walkey (1967) was unable to infect *Sialis lutaria* larvae with *N. rutili* eggs and, therefore, he considered only ostracods to be its true intermediate hosts. On the contrary, Lassiere and Crompton (1988) considered *S. lutaria* as a possible additional intermediate host of *N. rutili* and they also demonstrated experimental transmission of *N. rutili* from infected *S. lutaria* to raibow trout, *Oncorhynchus mykiss*. The seasonal occurrence and maturation of this acantho-

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cephalan was studied in *Gasterosteus aculeatus* in Great Britain by Walkey (1967) and in *Cyprinus carpio* in Czech Republic by Moravec (1984); the seasonal dynamics of *N. rutili* in *Phoxinus phoxinus* in Great Britain was dealt with by Bibby (1972).

This paper gives the results of long-term observations on *N. rutili* in the population of barbel, *Barbus barbus*, of the Jihlava River in the Czech Republic, carried out in 1992 and 1993. A detailed characterization of the barbel zone of this river, its fish and invertebrate faunas as well as data on the local population of barbel can be found in the papers by Peňáz (1977), Losos *et al.* (1980) and Zelinka *et al.* (1984).

MATERIALS AND METHODS

The seasonal occurrence and maturation of *N. rutili* were followed in barbel, *Barbus barbus* (L.) (length 14-51 cm, age 3-7 years) of the Jihlava River at its barbel zone near the village of Biskoupky (near Rosice u Brna) (the Danube River system) in South Moravia, Czech Republic; fish samples were taken at regular monthly intervals by means of an electric fishing machine from April 1992 until June 1993. Immediately after the transport of live fishes to the laboratory of the Institute of Parasitology in České Budějovice, they were examined for the presence of helminth parasites and their food components were recorded. In addition to 177 specimens of barbel, *Barbus barbus* (Table I), also 34 specimens of other fishes, belonging to 11 species, were examined; irregular samples of benthic and planktonic invertebrates were also collected and examined (for survey see Moravec and Scholz, 1994). All the recovered acanthocephalans were fixed in 4% formaldehyde and then stained with carmine and mounted as permanent preparations in Canada balsam.

RESULTS

THE DEFINITIVE HOST OF *N. RUTILI* AND ITS FOOD

Barbel, *B. barbus*, might be the main definitive host of *N. rutili* at this locality, in which both prevalence and mean intensity of infection are rather high. Moreover, the barbel is here by far the most abundant fish. Of other fishes examined from this locality, *N. rutili* was recorded in *Salmo trutta* m. *fario* (in 2 out of 3 examined; intensity 1), *Alburnoides bipunctatus* (in 1 out of 3; intensity 8), *Rutilus rutilus* (in 1 out of 4; intensity 2), *Leuciscus cephalus* (in 1 out of 11; intensity 3), and *Gobio gobio* (in 1 out of 6; intensity 3).

The character of the food of barbel was recorded monthly (for survey see Moravec and Scholz, 1994). It was found that there were almost no seasonal changes in the composition of food during the year and the diet of barbel consisted mainly of available larvae of aquatic insects (largely trichopterans, ephemeropterans and chironomids, less often other dipterans, plecopterans and megalopterans *[Sialis]* and other aquatic invertebrates-mollusks *[Pisidium, Ancylus]*, ostracods, gammarids), whereas the proportion of e.g. terrestrial insects or forage fish in their diet was negligible. Plant remnants were also frequently recorded. It was important to find that ostracods and *Sialis*, presumable main sources of *N. rutili* infection for barbel, formed significant components of the diet of these fish almost throughout the year (ostracods mainly in the period from December until June of the next year).

INVERTEBRATE HOSTS OF *N. RUTILI*

A total of 263 ostracod specimens belonging to the species *Candona candida* (O.F. Müller), *Cypridopsis vidua* (O.F. Müller), *Cyclocypris globosa* G.O. Sars and *Herpetocypris reptans* (Baird) were examined from this locality in addition to other aquatic invertebrates. No cystacanths of *N. rutili* were found.

An examination of 16 larval alder flies, *Sialis lutaria* (L.), revealed the presence of two *N. rutili* cystacanths in the body cavity of one of them (prevalence 6%), examined in September 1993. Both cystacanths represented male larvae and their morphology was practically identical with that in adult males from fishes. Their body (including the proboscis) was 0.78-1.07 mm long and 150-261 µm wide; the proboscis measured 120 × 120 µm in both specimens. The lengths of proboscis hooks were: first hooks 66-69 µm, second hooks 57-60 µm, third hooks 39 µm and fourth hooks 30 µm. Lemnisci were 315-519 µm long, the length of the proboscis receptacle was 105-180 µm. Both testes measured 66 × 54 µm in the smaller specimen and 120 × 78-96 µm in the larger one.

OCCURRENCE OF *N. RUTILI* IN BARBEL

Prevalence and intensity of infection : Of 177 *B. barbus* examined from this locality, 149 (prevalence 84%) were infected with *N. rutili*, the intensity of infection being 1-533 (mean 25) neoechinorhynchids per fish (see Table I).

Localization in the host : In the host's gut the acanthocephalans were localized along its whole length, largely in its anterior part, being most frequently concentrated in the first fourth of the intestine.

Year and month	N° of barbel examined	N° of barbel infected	Prevalence (%)	Intensity of infection (mean, range)	Body length of barbel in cm (mean, range)
1992					
April	12	11	92	55 (1-410)	34 (22-50)
May	14	11	79	59 (1-533)	32 (19-43)
June	11	8	73	95 (2-390)	37 (24-47)
July	12	7	58	6 (2-15)	30 (21-35)
August	12	10	83	6 (1-20)	28 (22-36)
September	12	11	92	9 (1-25)	26 (21-35)
October	13	10	77	10 (1-32)	29 (18-49)
November	12	10	83	16 (1-73)	29 (19-47)
December	15	13	87	20 (2-122)	33 (28-46)
1993					
January	13	11	85	39 (2-184)	37 (28-51)
February	10	10	100	39 (1-189)	35 (30-51)
March	10	10	100	41 (2-115)	35 (24-45)
April	10	9	90	27 (1-40)	29 (14-35)
May	10	8	80	7 (1-14)	35 (30-40)
June	11	10	91	11 (2-25)	30 (16-42)
Total	177	149	84	25 (1-533)	28 (14-51)

Table I. – Survey of *Barbus barbus* examined from the Jihlava River and their infection with *Neoechinorhynchus rutili*.

Infection in relation to the size of host : A survey of barbel examined and their infection with *N. rutili* is given in Table I. Figure 1 shows that the acanthocephalan infections in barbel are distinctly dependent on the body size (age) of the hosts. The smallest barbel specimen harbouring *N. rutili* measured 18 cm (age 3 years) and the largest one 51 cm (age 7 years). The acanthocephalans occurred in all size-groups of barbel examined. It is evident from Fig. 2 that the values of both the prevalence and the mean intensity of infection distinctly increased with the body length of hosts to reach their maxima in the size-group of the largest fish (body length above 40 cm, age 6-7 years).

SEASONAL CHANGES IN PREVALENCE AND MEAN INTENSITY OF INFECTION

A survey of prevalence, intensity and mean intensity of *N. rutili* infection in barbel in individual months is given in Table I. It is apparent from Fig. 2 that this parasite occurred in barbel throughout the year, with the prevalence attaining 100% in February and March. The lowest value of prevalence was found in July when also a sudden decrease of mean intensity was recorded in 1992. Then the mean intensity values gradually increased to attain higher values only in winter (January, March) in 1993 and then again decreased. These fluctuations appear to be closely associated with the parasite's seasonal cycle of maturation.

SEASONAL CHANGES IN THE MATURATION OF *N. RUTILI*

The state of acanthocephalan maturity in individual monthly samples from barbel is shown in Fig. 3. The first group of parasites includes the juvenile forms of *N. rutili* (body length of males below 2 mm, females about 3 mm, with compact ovary), the second group is males, the third group is young females with ovarian balls and with or without immature eggs, and the fourth one is gravid females containing mature eggs (shelled acanthors).

It is obvious from Fig. 3 that the parasite exhibited a distinct annual cycle of maturation when egg-producing females were present only from December until August of the next year, but mainly in the spring and summer (May-July); they were completely absent in the period from September to November.

The recruitment of the parasite occurred nearly all the year round, but mainly in the early spring (March - April) and in the late summer and autumn (August - September) (Fig. 3). The males and young females (with or without immature eggs) were present throughout the year with their lowest proportions in samples being in March and September, when the parasite's larvae predominated.

The present data show a clearly pronounced seasonal maturation cycle of *N. rutili* in *B. barbus* in this locality.

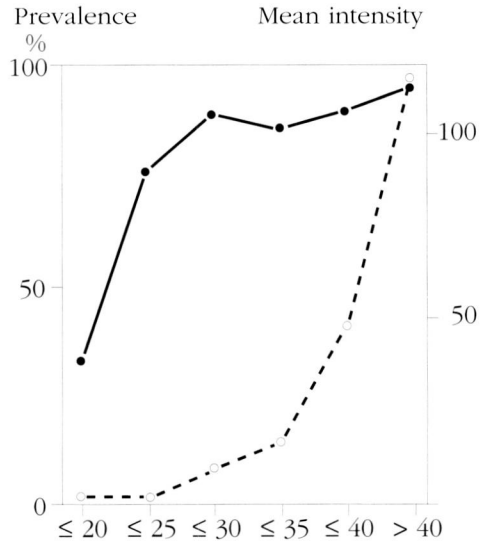

Fig. 1. – Relationship of prevalence (– – – –) and mean intensity (- - - -) of *Neoechinorhynchus rutili* infection to body length of barbel.

DISCUSSION

Like in other congeneric species, the life cycle of *Neoechinorhynchus rutili* requires an obligate intermediate host, various species of Ostracoda, whereas other invertebrates (leeches, alder flies, crayfish) in which *N. rutili* larvae were observed (Robin, 1871; Villot, 1885; Merrit and Pratt, 1964; Lassiere and Crompton, 1988; present data) may apparently serve only as paratenic hosts.

Walkey (1967) was not able to experimentally infect the alder fly, *Sialis lutaria*, with *N. rutili* and he concluded that *S. lutaria* was not a likely intermediate host of this parasite. He suggested that *S. lutaria* was most likely to act as a paratenic host, acquiring its infection through feeding on infected ostracods. In Lassiere and Crompton's (1988) opinion, "the view that *S. lutaria* can serve as an intermediate host for *N. rutili* must be considered in the light of knowledge of the relationship between *N. rutili* and ostracods as the intermediate hosts", i.e. it is important to discover exactly how *N. rutili* becomes established in *S. lutaria*. Nevertheless, in view of the fact that these insect larvae are extremely predacious (Kimmins, 1962) and because they frequently harbour also infective larvae of fish cestodes of the genus Proteocephalus (Vojtková and Roubková, 1990; Kennedy *et al.*, 1992; Scholz and Moravec, 1993) developing through plankton crustaceans (copepods) as intermediate hosts, we consider alder flies *(Sialis)* to be rather paratenic than true intermediate hosts of *N. rutili*. In view of a relatively rare occurrence of *Sialis* larvae in the locality, the significance of alder flies as a source of *N. rutili* infection for barbel seems to be limited. Zelinka *et al.* (1984) reported another species of *Sialis, S. fuliginosa* Pictet from this locality; apparently, this species may serve as a paratenic host of *N. rutili* too.

The following ostracod species have so far been reported as the intermediate hosts of *N. rutili* : *Candona angulata, C. candida, C. neglecta, Cypria ophthalmica, C. turneri* and *Cyclocypris laevis* (Brady, 1910; Jarecka, 1956; Styczynska, 1958; Merrit and Pratt, 1964; Walkey, 1967; Valtonen, 1979). Of them, *C. candida* was found to occur in the Jihlava River along with a few additional ostracod species of other genera including *Cyclocypris*. It is highly probable that these crustaceans might serve as intermediate hosts of *N. rutili* in this locality despite the fact that no cystacanths of *N. rutili* were recorded in them. This was apparently due to a small number of ostracods examined. Since ostracods (mainly the species *Herpetocypris reptans*) frequently occurred in the food of barbel, they might play an important role in the transmission of this parasite to barbel here. According to Prof. F. Kubíček (pers. comm.), in this locality the ostracods live mainly near the river banks, where regular fluctuations of the water level occur, and also in the growths of *Batrachium* where barbel frequently occur.

The present data indicate a distinct seasonal maturation cycle of *N. rutili* in this locality; its seasonal pattern is as follows: The mature eggs of *N. rutili* are laid mainly in the period from May until July when the water temperature ranged between 11-16°C. After oviposition the adult acanthocephalans, mainly females, are gradually expelled from the host which reflects in the decrease of prevalence and mean intensity values, attaining their minima in July (Fig. 3). In August only a small number of gravid females with few mature eggs survived in barbel, whereas these completely disappeared from the host from September until November.

The eggs get to the water along with the host's excrements where they are eaten by ostracod intermediate hosts. In them the parasite continues to develop up to the cystacanth stage, which is already infective to fishes. At this time the water temperature in the locality is about 15-20°C and it can be estimated that the development in the intermediate host lasts about 3-4 weeks (according to Walkey [1967] 20-30 days at 18°C). During August and September this development in the intermediate host is mostly finished and, consequently, new *N. rutili* infections are acquired by fish in these months, which reflects in a considerably increased proportion of juvenile acanthocephalans in monthly samples and in a distinct decrease of prevalence. Some of these newly obtained acanthocephalans can develop in barbel up to maturity during

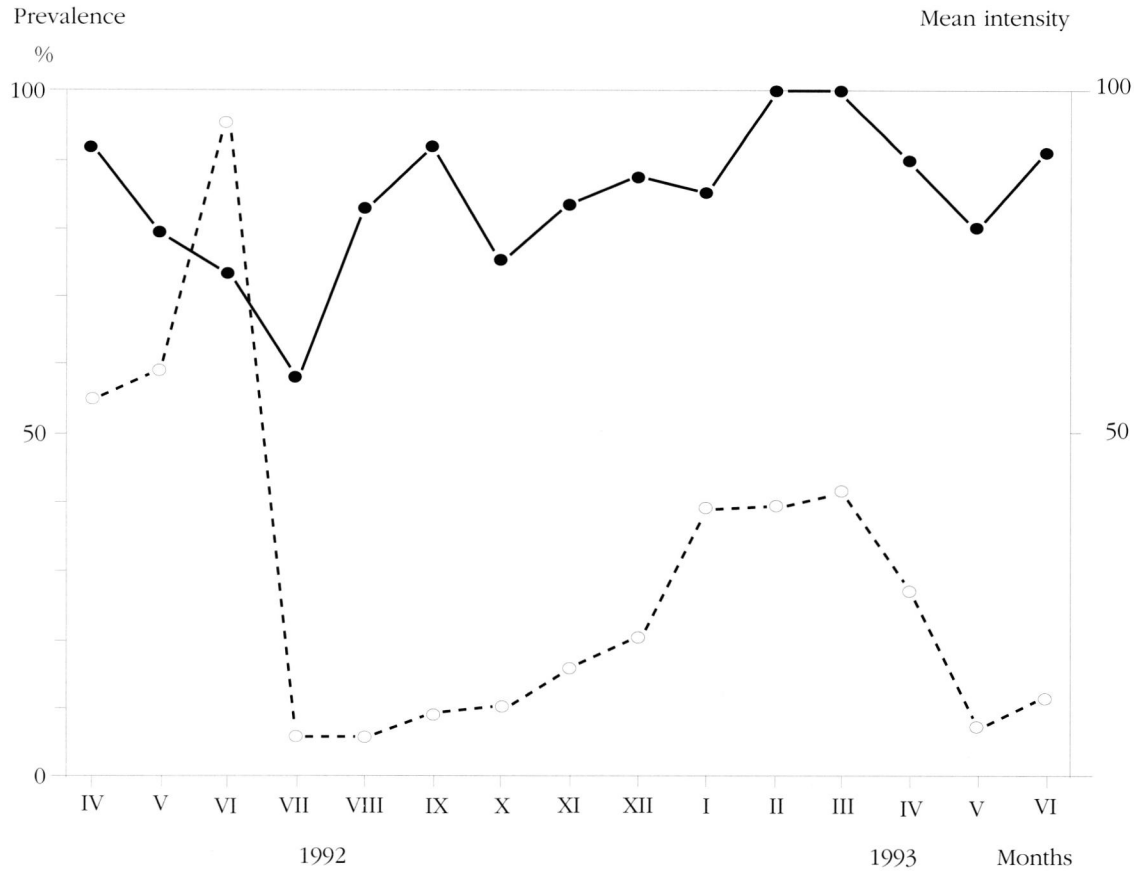

Fig. 2. – Seasonal variation of prevalence (– – – –) and mean intensity of infection (- - - -) of *Neoechinorhynchus rutili* in barbel of the Jihlava River.

autumn and a small number of females can even produce eggs during winter months (December-February) (Fig. 3). In the period from October until January new infections are acquired by fish in a much lesser extent, or the acanthocephalan larvae obtained by the host in the autumn can survive in it without any further development. Another period when new *N. rutili* infections are mainly acquired by fish is from February to March (apparently associated with increasing water temperature), extending sometimes up to May (Fig. 3); this reflects also in the higher values of prevalence and mean intensity in February and March (Fig. 2). New infections in fish are rare from June until July (or sometimes earlier) which may be related either with the seasonal changes in populations (life cycles) of the ostracod intermediate hosts or with the increased 12 mortality of infected intermediate hosts in the warmer months.

The larval and mature acanthocephalans that survived in fish from the foregoing year and the larvae acquired at the beginning of the following year quickly develop in barbel during spring months so that the percentage of egg-producing females is relatively high during May-July.

Walkey (1967) observed an annual maturation cycle of *Neoechinorhynchus rutili* in *Gasterosteus aculeatus* in Great Britain, manifested, however, only quantitatively; the gravid females with mature eggs were present in fishes all the year round, but mostly in the late spring and early summer, whereas new infections were acquired during the whole year, but largely from summer to winter. According to Tesařík (1970), the oviposition of *N. rutili* in *Cyprinus carpio* of the South Bohemian ponds in the Czech Republic occurs in the period from March until July, whereas Moravec (1984) reported a more strictly seasonal maturation cycle of this parasite in the same host species (carp) in the North Bohemian pond Mácha Lake, when gravid egg-producing female acanthocephalans were present only in May. The maturation cycle of *N. rutili* in barbel of the Jihlava River, as observed in this study, is less clear-cut than observed by Tesařík (1970) or Moravec (1984) but, on the other hand, is more pronounced than that reported by Walkey (1967). These differences appear to be induced principally by the temperature regime in the locality (Moravec 1984).

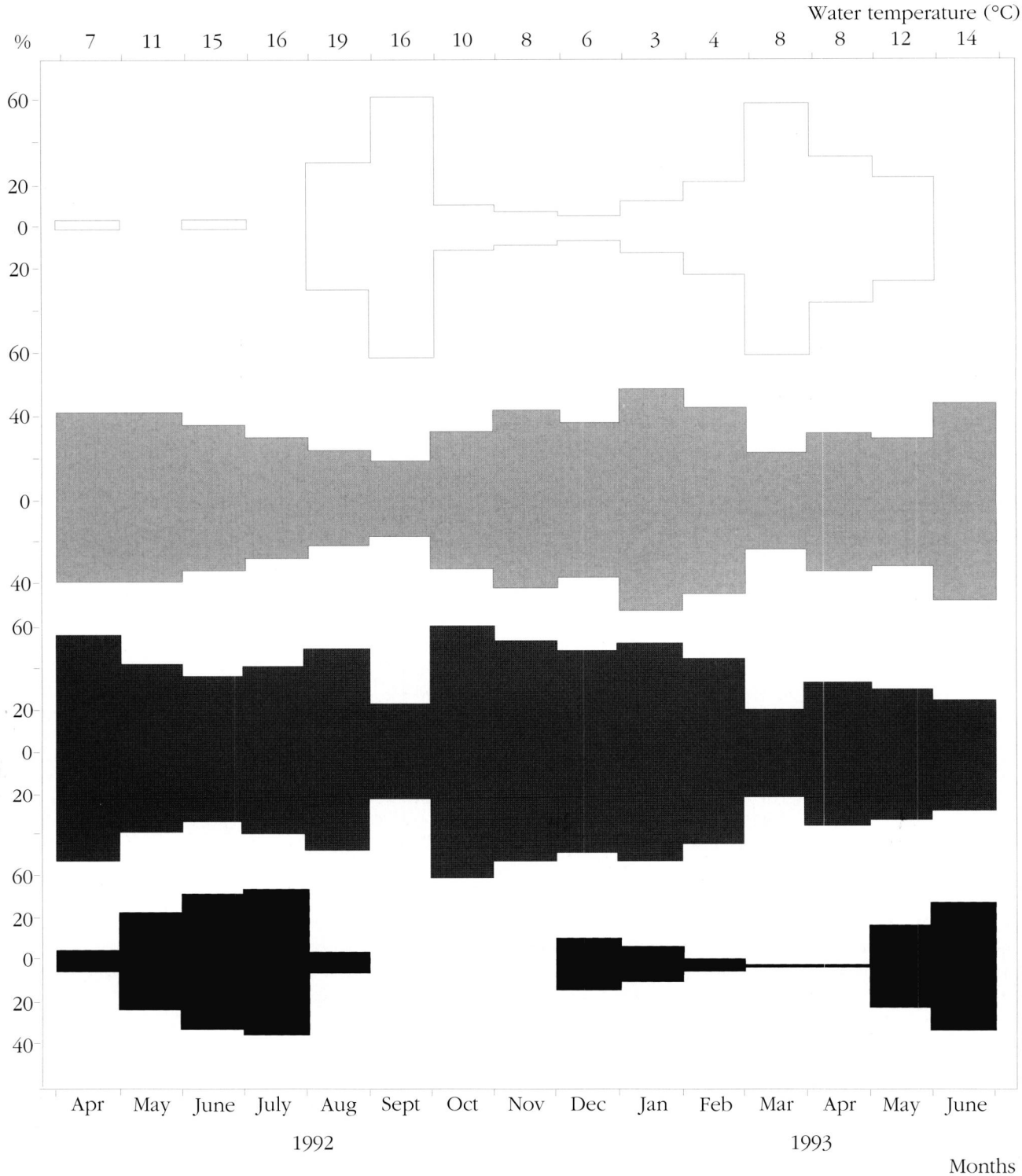

Fig. 3. – Monthly changes in samples of *Neoechinorhynchus rutili* observed in barbel in the Jihlava River. Data are expressed as percentages of the total number of acanthocephalans found per month: juveniles (unsheaded), males (stippled), females with immature eggs (hatched), and females with mature eggs (black).

In contrast to observations by Walkey (1967) and Bibby (1972), who reported the prevalence and mean intensity of *N. rutili* to be decreased in the winter and increased in the summer, the results of the present study are quite opposite : the highest prevalence was found in February-March and the lowest in July, whereas the lowest mean intensity in July. The highest values of *N. rutili* prevalence in winter were also recorded by Valtonen (1979) and Valtonen and Crompton (1990) in *Coregonus widegreni* and *Gymnocephalus cernuus* in the Bothnian Bay in Finland. Similar data were obtained by Moravec (1984), who found the maximum values of *N. rutili* prevalence and mean intensity in carp of the Mácha Lake pond in a cool period from autumn until early spring; since May a sudden decline of prevalence and later also of mean intensity took place and this situation lasted during the whole summer. A similar seasonal periodicity was observed by Eure (1976) in a congeneric North American species *N. cylindratus*; the author believed that the drastical decrease in the intensity in summer was related to the temperature-dependent host rejection response.

In this study, a distinct dependence of the prevalence and mean intensity of *N. rutili* infection on the body size (age) of barbel was recorded, when the highest values were found in the largest fish. The same was observed by Walkey (1967) and Bibby (1972) in the hosts *Gasterosteus aculeatus* and *Phoxinus phoxinus*, respectively. In contrast, Moravec (1984) observed in *Cyprinus carpio* of the pond Mácha Lake that the prevalence of *N. rutili* increased with the fish size but the mean intensity first increased to attain its maximum in carp 20-25 cm long and decreased again in the largest carp. The author explains it so that in smaller fishes the consumption of food is generally increasing with their growth which results in their increasing infection by this parasite. However, in larger-sized fishes (large *C. carpio*) the proportion of ostracods (intermediate hosts of *N. rutili*) in their food is gradually decreasing, this leading to the decrease in the intensity of infection. The fact that in barbel of the Jihlava River the maximum value of the mean intensity of *N. rutili* was found in the largest fish can be explained by the presence of *Sialis* larvae (another source of *N. rutili* infection) in the locality; it can be expected that, in contrast to ostracods, the proportion of larval *Sialis* in the barbel's food is practically the same in all size-groups of barbel studied.

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